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Dario Angelone, Sanathana Konugolu Venkata Sekar, Walter Messina, Andrew Matheson, Ahmet T. Erdogan, Robert K Henderson, Ray Burke, Stefan Andersson-Engels, "Time resolved SPAD micro-camera probe for wide-field FLIm in microendoscopy," Proc. SPIE 12368, Advanced Biomedical and Clinical Diagnostic and Surgical Guidance Systems XXI, 1236807 (6 March 2023); doi: 10.1117/12.2648340

SPIE.

Event: SPIE BiOS, 2023, San Francisco, California, United States

Time resolved SPAD micro-camera probe for wide-field FLIm in microendoscopy

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ABSTRACT

We propose a handheld single photon avalanche diode (SPAD) micro-camera probe for wide-field *in-vivo* fluorescence lifetime imaging (FLIm) applications. The presented probe includes a novel 3D stacked 1.4 mm × 1.4 mm SPAD array, an integrated excitation light source, and imaging optics. The spatial and temporal performance of the integrated system was characterised using a USAF test target and range of fluorescence lifetime beads.

Keywords: fluorescence lifetime imaging, FLIm, SPAD, micro-camera

1. INTRODUCTION

Recently, FLIm has proved to be an effective method for label-free intraoperative tumour margin assessment both *ex vivo*[1,2] and *in vivo*.[3] *In vivo* FLIm probes typically employ point scanning to recover lifetime images and thus far, there are few examples of wide-field *in vivo* FLIm and even fewer where sensing occurs at the distal end of the probe.[4,5] Wide-field *in vivo* FLIm would have the benefit of capturing lifetime information from the entire field of view at the same time. Developments in the miniaturisation of SPAD arrays with time resolving capability make their use in wide-field *in vivo* FLIm feasible. Here a probe using a miniaturised SPAD array with half-inch diameter optics and an integrated excitation source is presented. The system is characterised in terms of spatial resolution and temporal performance.

2. MATERIALS AND METHODS

The probe design uses an epi-illumination scheme as illustrated in Fig. 1a with the excitation light coupled into an optical fibre which is connected to the probe. A long-pass dichroic mirror and filter (Semrock FF506-DI03-25X36 and FF01-515/LP-25) are used to direct the excitation light towards the sample and filter out elastically scattered light, respectively. Two achromatic doublets with f =19 mm are used, one to collect the light from the sample, the second to re-focus it onto the sensor. The sensor for the probe is a 128 × 120 SPAD array with dimensions of 1.4 mm × 1.4 mm implemented in STMicroelectronics 40 nm/90 nm 3D-stacked BSI CMOS process with 8 µm pixels and a 45% fill factor. This sensor is described in detail by Erdogan et al.,[6] in which its *ex vivo* FLIm capability with biological samples was demonstrated using a 10X objective mounted on a fluorescence microscope system.

The spatial resolution was quantified by imaging a USAF resolution target. The temporal system performance was evaluated by measuring the instrument response function (IRF) and through measurements of lifetime-encoded fluorescent polymer beads (PolyAn GmbH). The beads were measured suspended in a solution in a well plate; therefore there was no spatial variation in lifetime to be resolved so all pixel values were binned for the fitting process. Intensity and lifetime images of a plant sample were also captured. Lifetime fitting was carried

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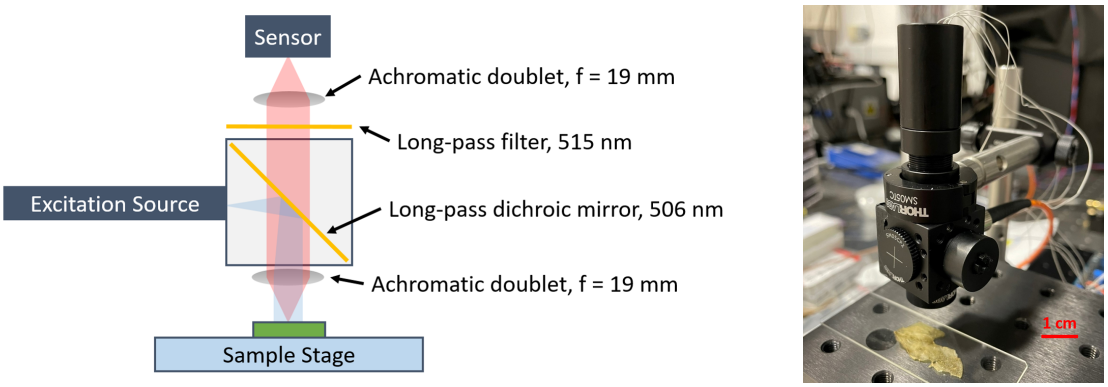
Advanced Biomedical and Clinical Diagnostic and Surgical Guidance Systems XXI,
edited by Caroline Boudoux, James W. Tunnell, Proc. of SPIE Vol. 12368,
1236807 · © 2023 SPIE · 1605-7422 · doi: 10.1117/12.2648340



(a) (b)

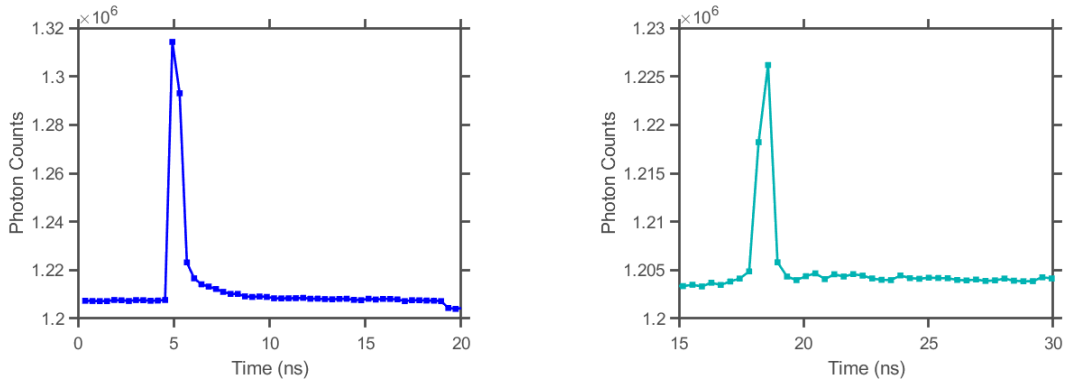
Figure 1: A basic schematic (a) and a photograph (b) of the miniaturised wide-field FLIm microscope.

out using eight time bins with a width of 390 ps each. The fluorescent beads were excited at 488 nm using a Fianium SC450 supercontinuum source and a monochromator and the plant sample was excited at 405 nm using a Becker and Hickl pulsed laser diode. Both sources were operated at a repetition rate of 20 MHz. The IRF was measured with both sources by placing a non-fluorescent white standard in the sample plane.

3. RESULTS

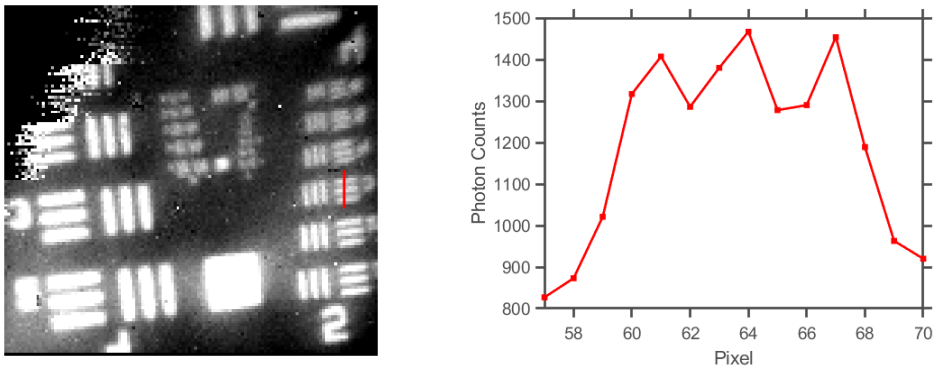
The IRF at 405 nm and 488 nm are show in Fig. 2 and are found to have a full width half maximum (FWHM) of 0.74 ns and 0.62 ns, respectively. An intensity image of a USAF resolution target captured with white light illumination is shown in Fig. 3a. The smallest resolved feature is Group 5 element 3 which has a line width of 12.4 µm; the pixels along this element are indicated by the red line in Fig. 3a and the pixel values are plotted in Fig. 3b. A race condition between pixel read and reset signals has caused a corner of the array to be insensitive to light[6] as can be seen in Fig. 3a; the images presented in Fig. 5 have been cropped to exclude this area.

The fluorescence decay curves from the lifetime-encoded bead measurements are presented in Fig. 4 with the quoted and measured lifetimes indicated in the legend. The different types of beads are clearly differentiated though there is a slight underestimation of the lifetime compared to the quoted value. Intensity and lifetime images of a leaf sample are shown in Fig. 5 demonstrating the capability of the miniaturised microscope to capture wide-field lifetime images of autofluorescence from biological samples.



(a) (b)

Figure 2: IRF measured with a 405 nm Becker and Hickl pulsed diode laser (a) and a Fianium supercontinuum laser at 488nm (b) found to have a FWHM of 0.74 ns and 0.62 ns, respectively.



(a) (b)

Figure 3: (a) Intensity image of a USAF resolution target captured with white light illumination to characterise the system imaging performance. (b) Plot of pixel values along the red line indicated in the intensity image left. Group 5 element 3 on the resolution target has been resolved and has a line width of 12.4 µm.

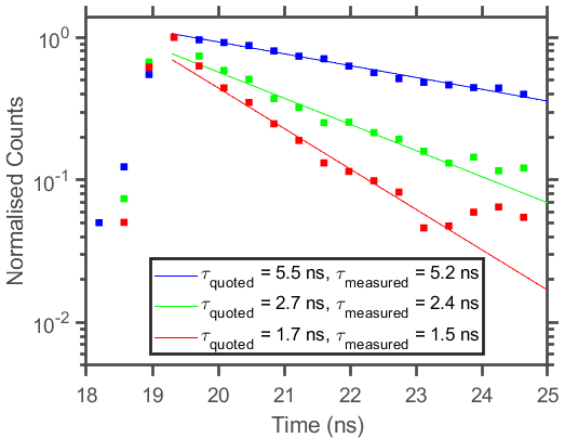
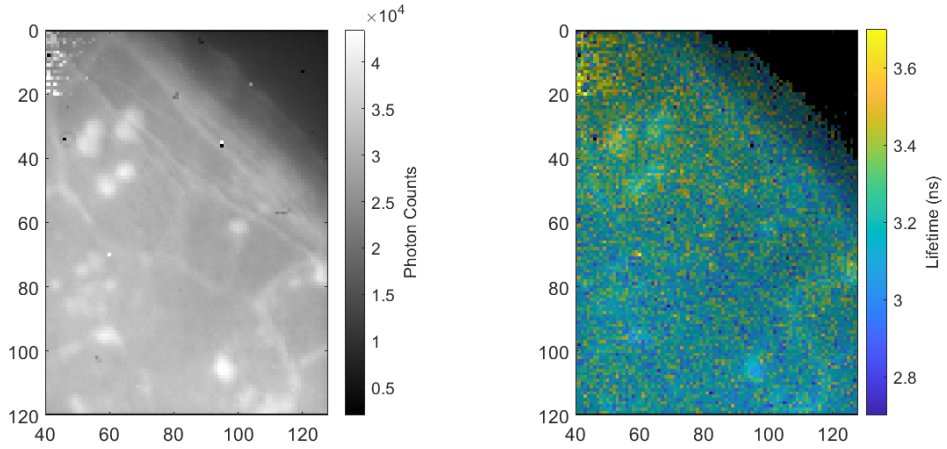


Figure 4: Scatter plot of the measured fluorescence decay for the different lifetime encoded beads with the fit indicated by the line plots. Fitting for all beads was carried out using 8 time bins with a width of 390 ps each.



(a) (b)

Figure 5: Fluorescence intensity (a) and lifetime (b) images of a leaf sample captured with excitation at 405 nm.

4. CONCLUSION

A handheld SPAD micro-camera probe for wide-field *in-vivo* FLIm applications including a novel 3D stacked 1.4 mm × 1.4 mm SPAD array, an integrated excitation light source, and imaging optics has been presented. The presented probe has a spatial resolution of 12.4 µm and has been shown to successfully differentiate polymer bead samples with a range of fluorescence lifetimes. An intensity and lifetime image of a plant sample has also been presented.

ACKNOWLEDGMENTS

We gratefully acknowledge funding from the EU H2020 MSCA-ITN project "Photonics for Healthcare: multiscale cancer diagnosis and therapy" (PHAST-ETN), grant agreement no. 86018; Science Foundation Ireland (SFI/15/RP/2828 and SFI-12/RC/2276_P2); and the PROTEUS project (http://proteus.ac.uk), Grant no: EP/K03197X/1.

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